

US007777885B2

(12) **United States Patent**
Coumans et al.

(10) **Patent No.:** **US 7,777,885 B2**
(45) **Date of Patent:** **Aug. 17, 2010**

(54) **DIAGNOSTIC IMAGING DEVICE FOR THE ANALYSIS OF CIRCULATING RARE CELLS**

(75) Inventors: **Frank A. W. Coumans**, GD Stein, NE (US); **Arthur G. Marlin**, Willow Grove, PA (US); **Frank P. Modica**, Princeton, NJ (US); **John V. Verrant**, Solebury, PA (US)

(73) Assignee: **Veridex, LLC**, Raritan, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 186 days.

(21) Appl. No.: **11/701,765**

(22) Filed: **Feb. 2, 2007**

(65) **Prior Publication Data**

US 2007/0153280 A1 Jul. 5, 2007

(51) **Int. Cl.**
G01N 21/25 (2006.01)

(52) **U.S. Cl.** **356/417**; 435/2; 435/6; 435/7.23

(58) **Field of Classification Search** 356/417; 435/2, 6, 7.23, 7.24, 69.3, 70.2, 172
See application file for complete search history.

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Primary Examiner—Gregory J Toatley, Jr.
Assistant Examiner—Iyabo S Alli

(57) **ABSTRACT**

The present invention provides a system for imaging circulating tumor cells from blood after enrichment. The system is designed to provide optimum use in a clinical laboratory setting with minimum laboratory bench top space. Operator intervention is minimized compared to other analytical methodologies. The system is useful in the enumeration and identification of target cells in a sample specimen for screening and detection of early stage pre-metastatic cancer, monitoring for disease remission in response to therapy and selection of more effective dose regimens or alternative therapies for individual patients.

10 Claims, 11 Drawing Sheets

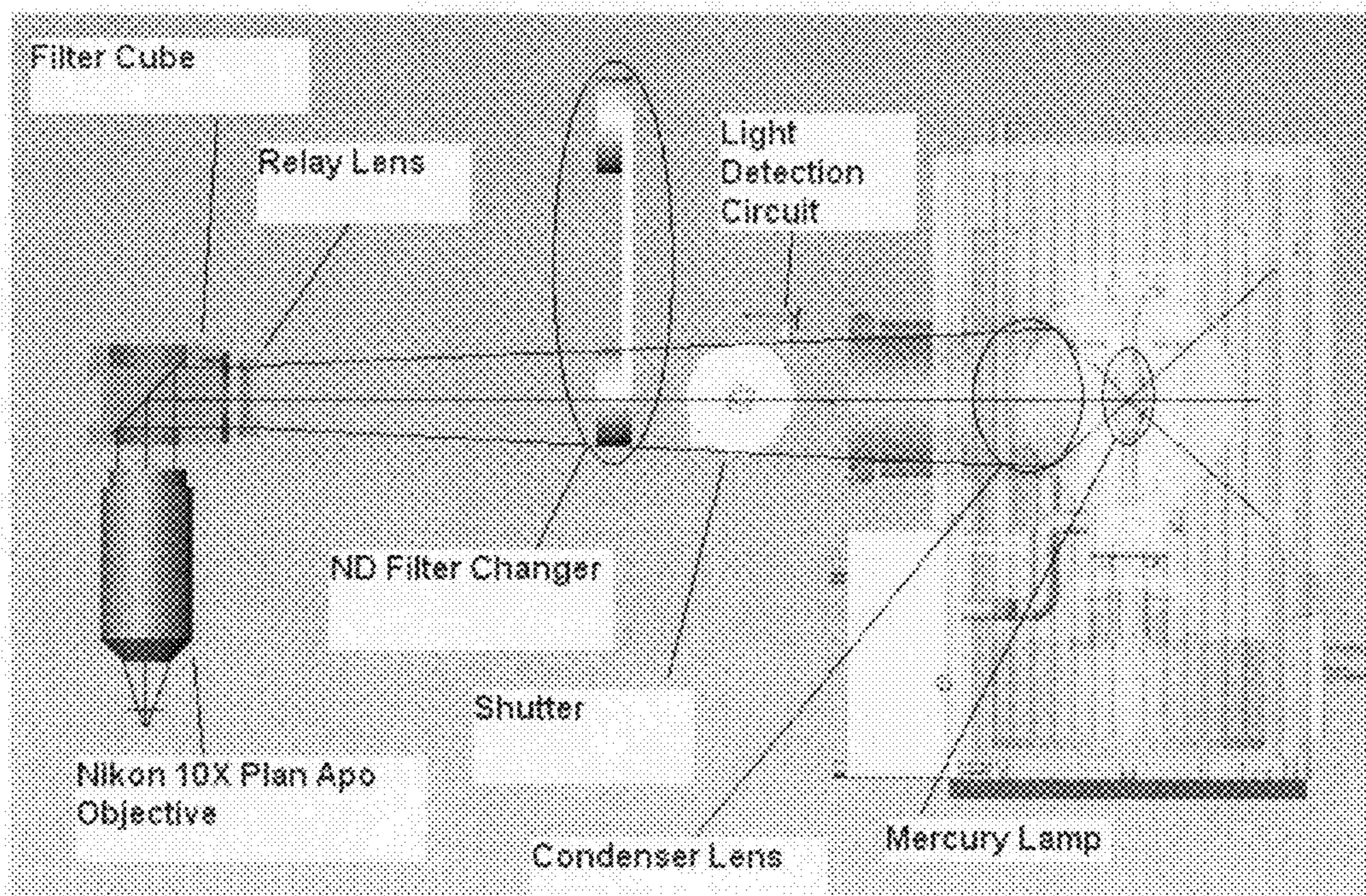
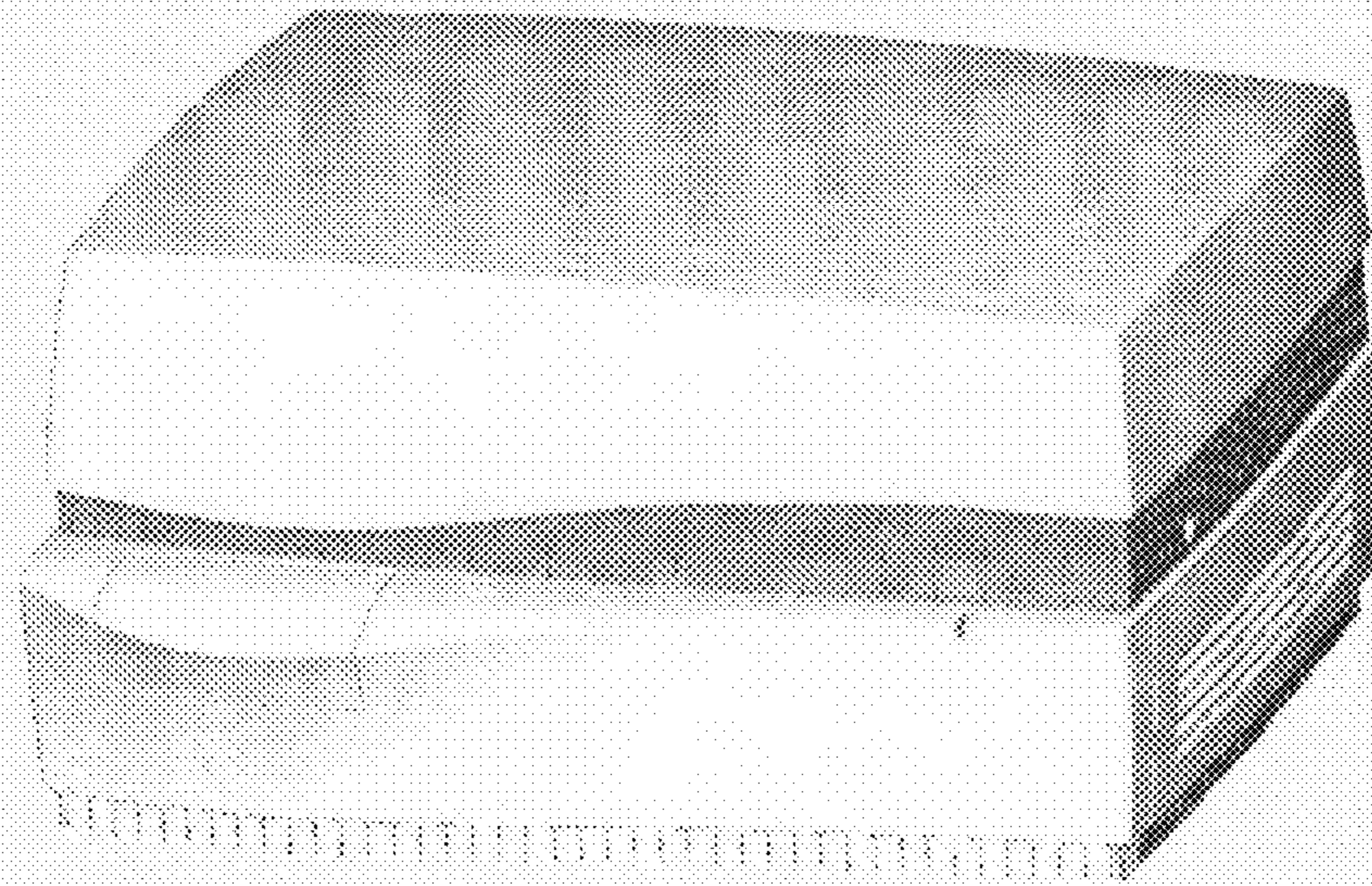


Figure 1

Panel-A



Panel-B

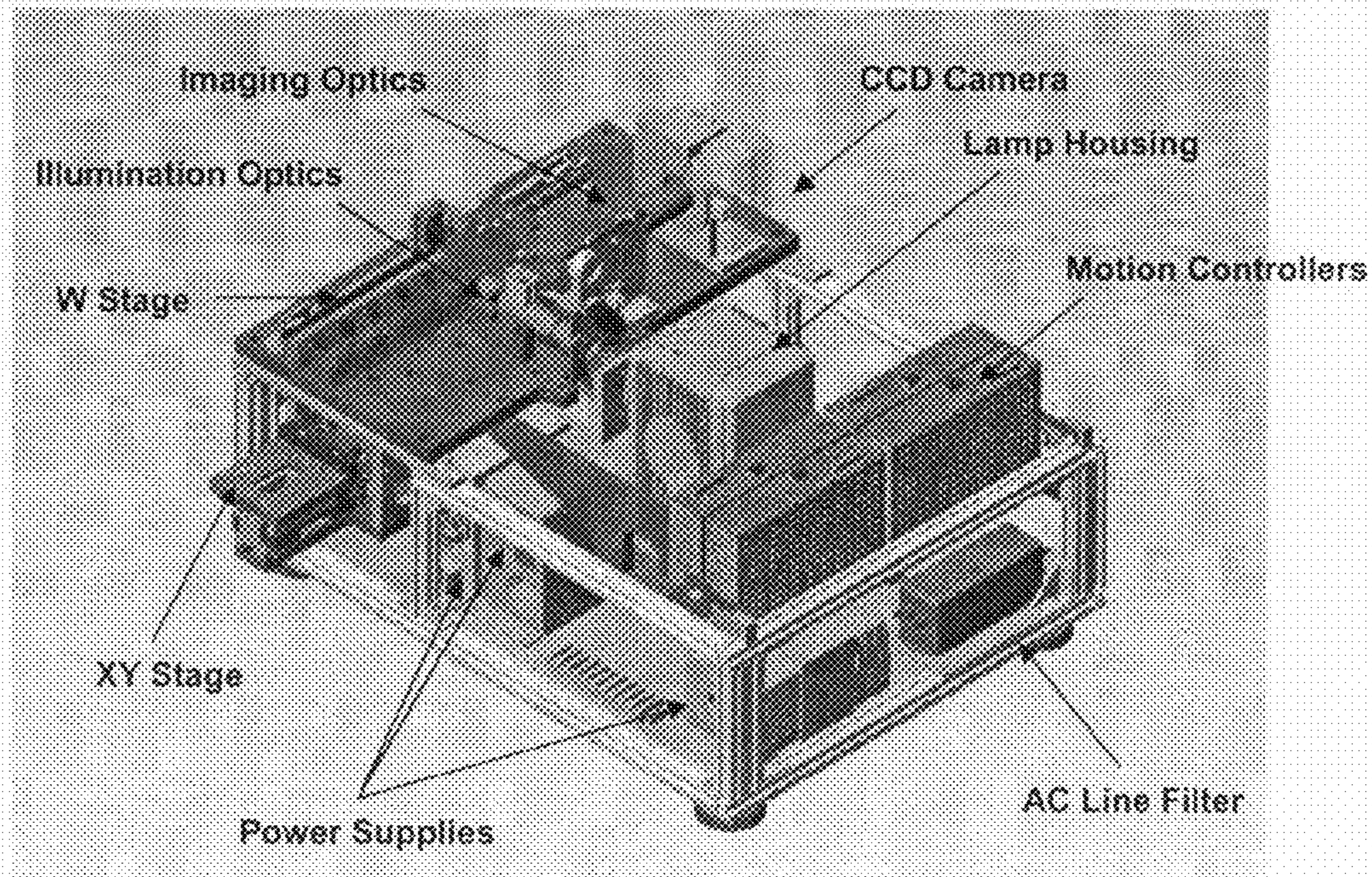


Figure 2

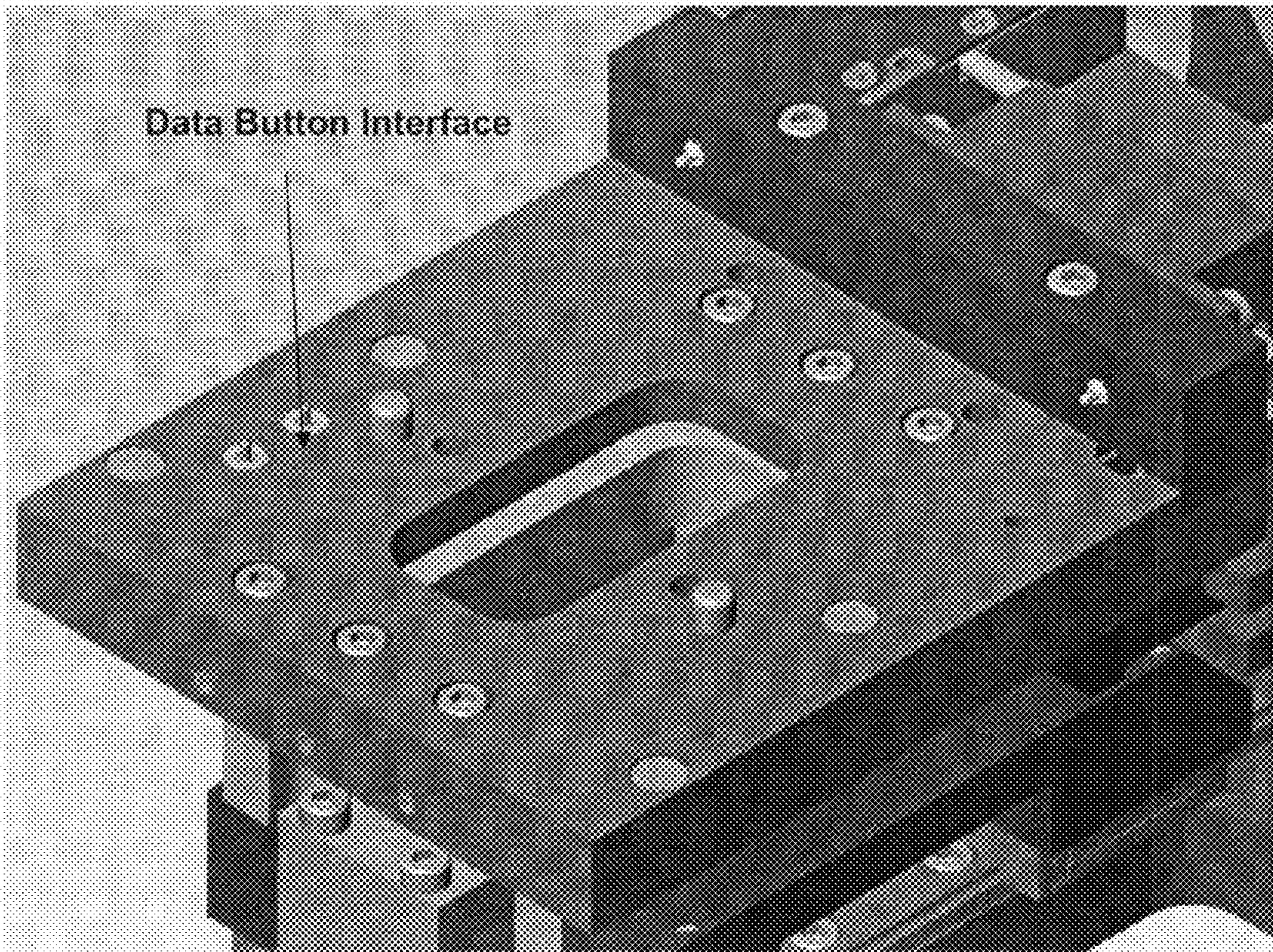


Figure 3

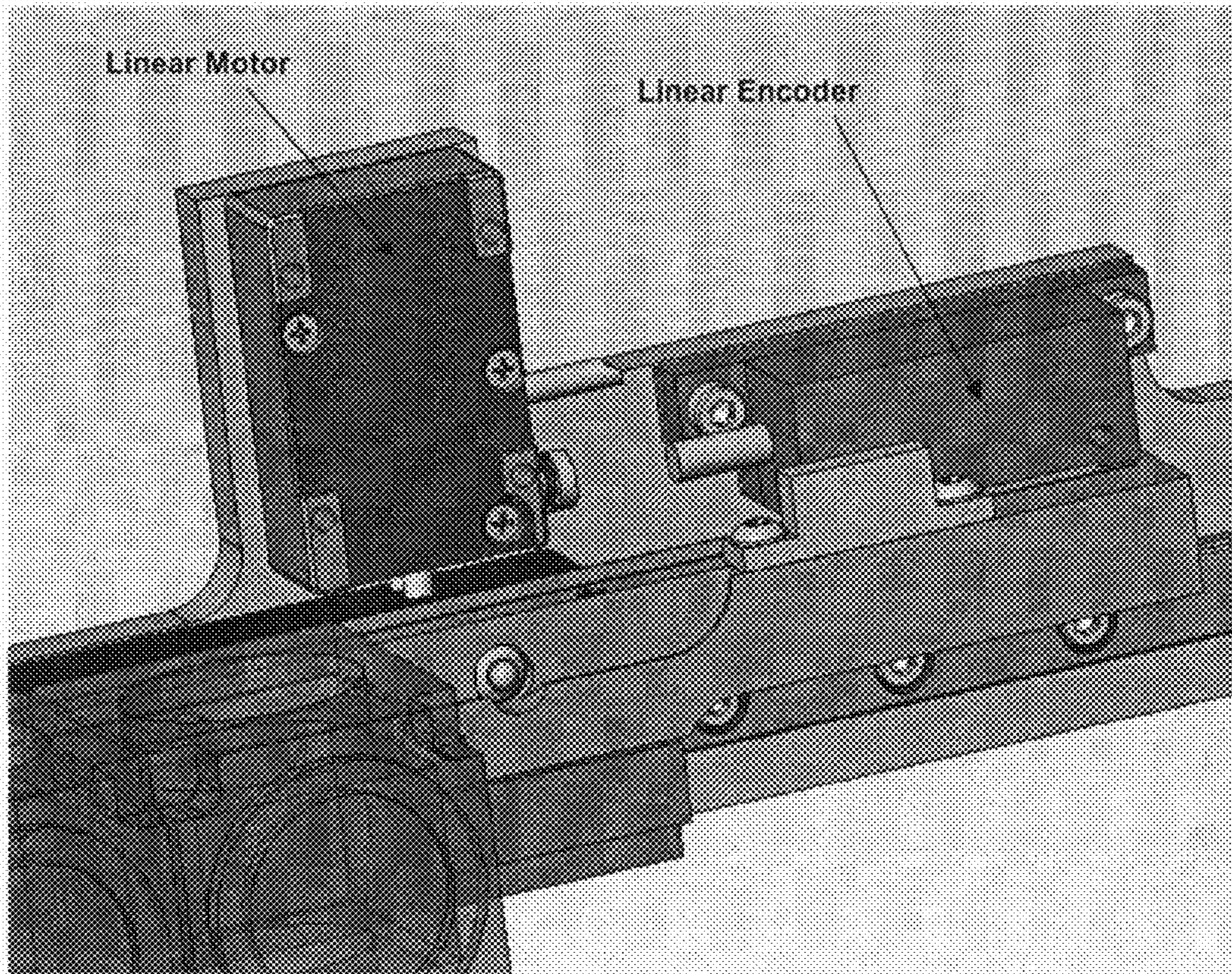


Figure 4

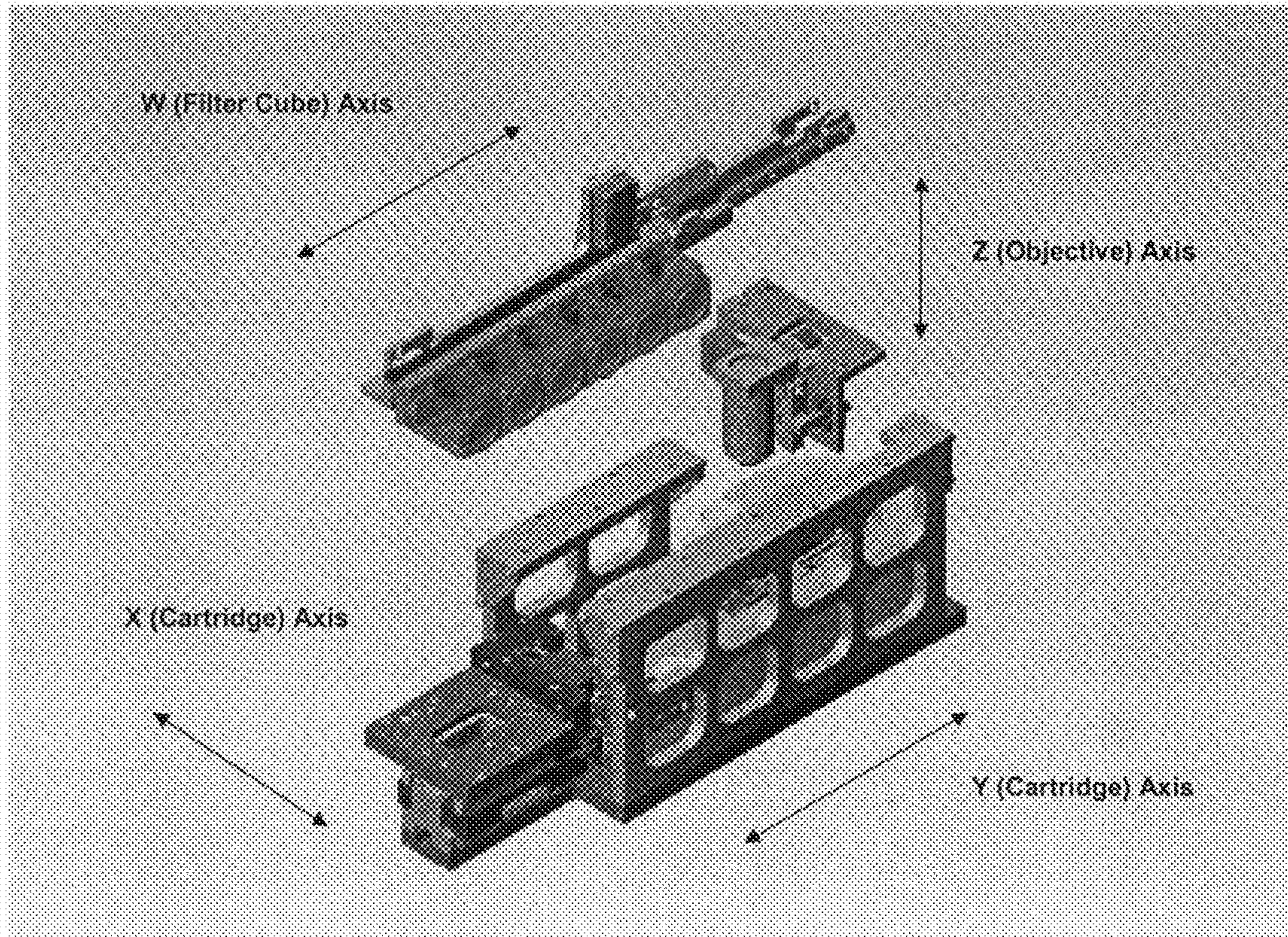


Figure 5

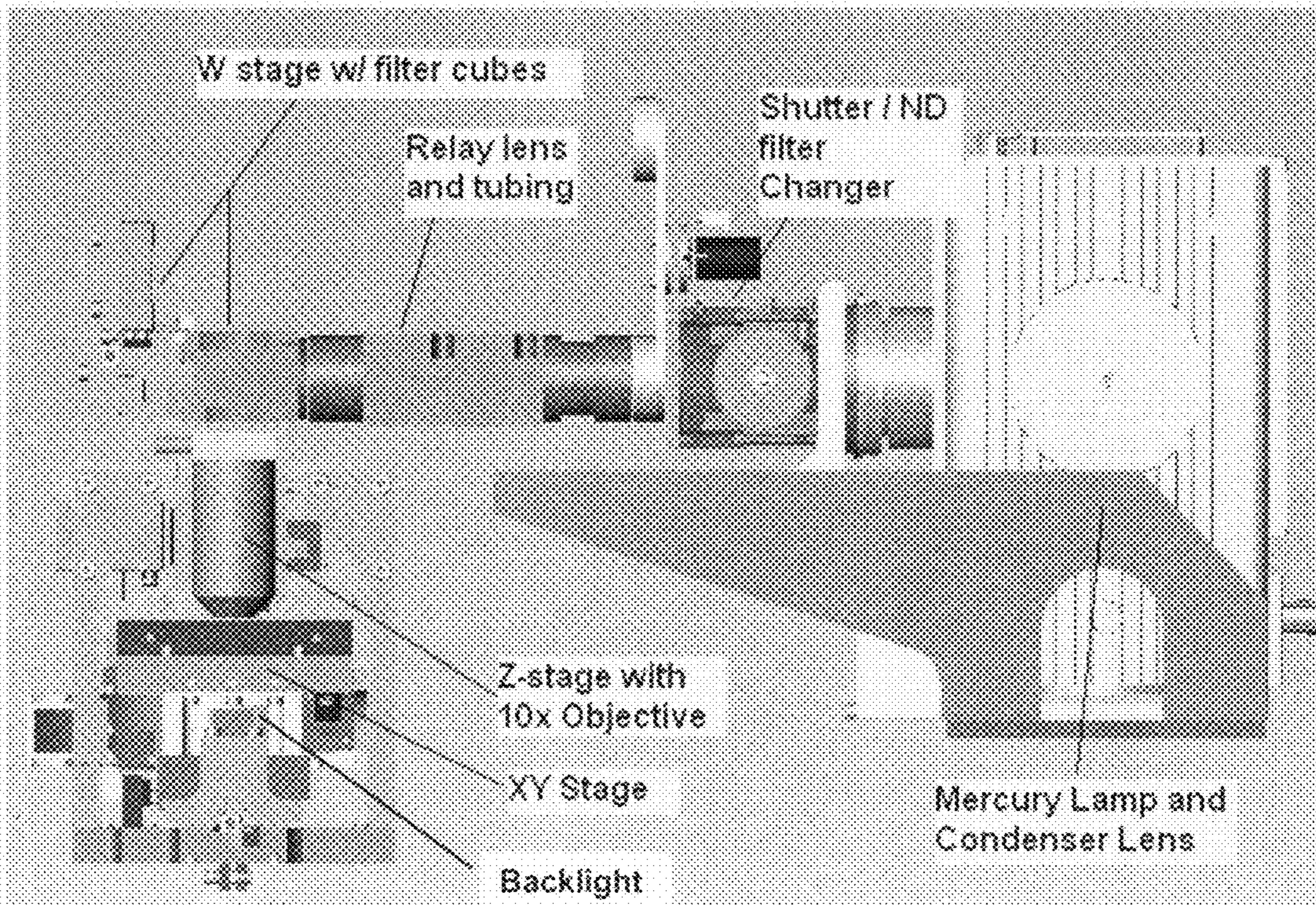


Figure 6

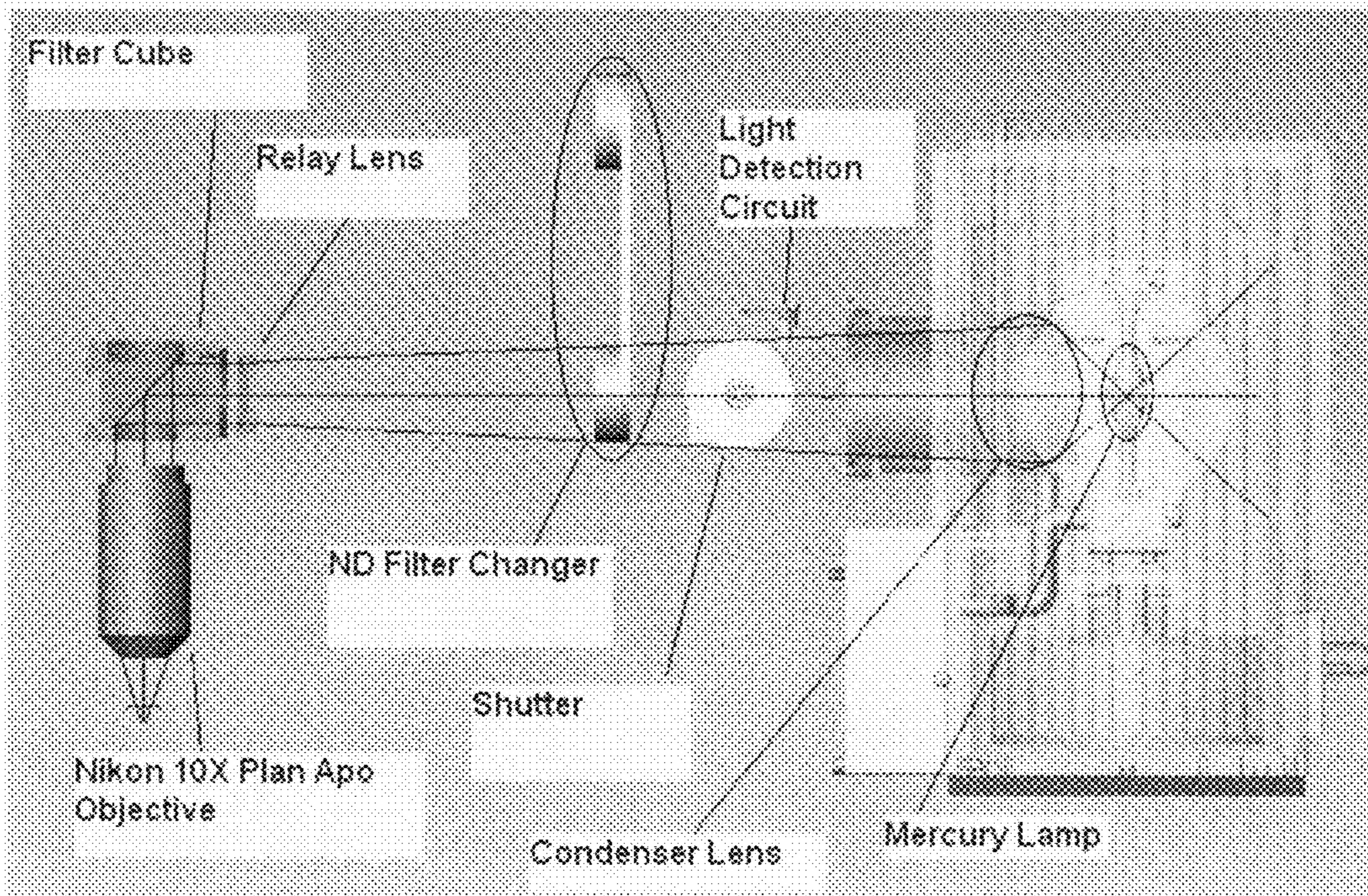


Figure 7

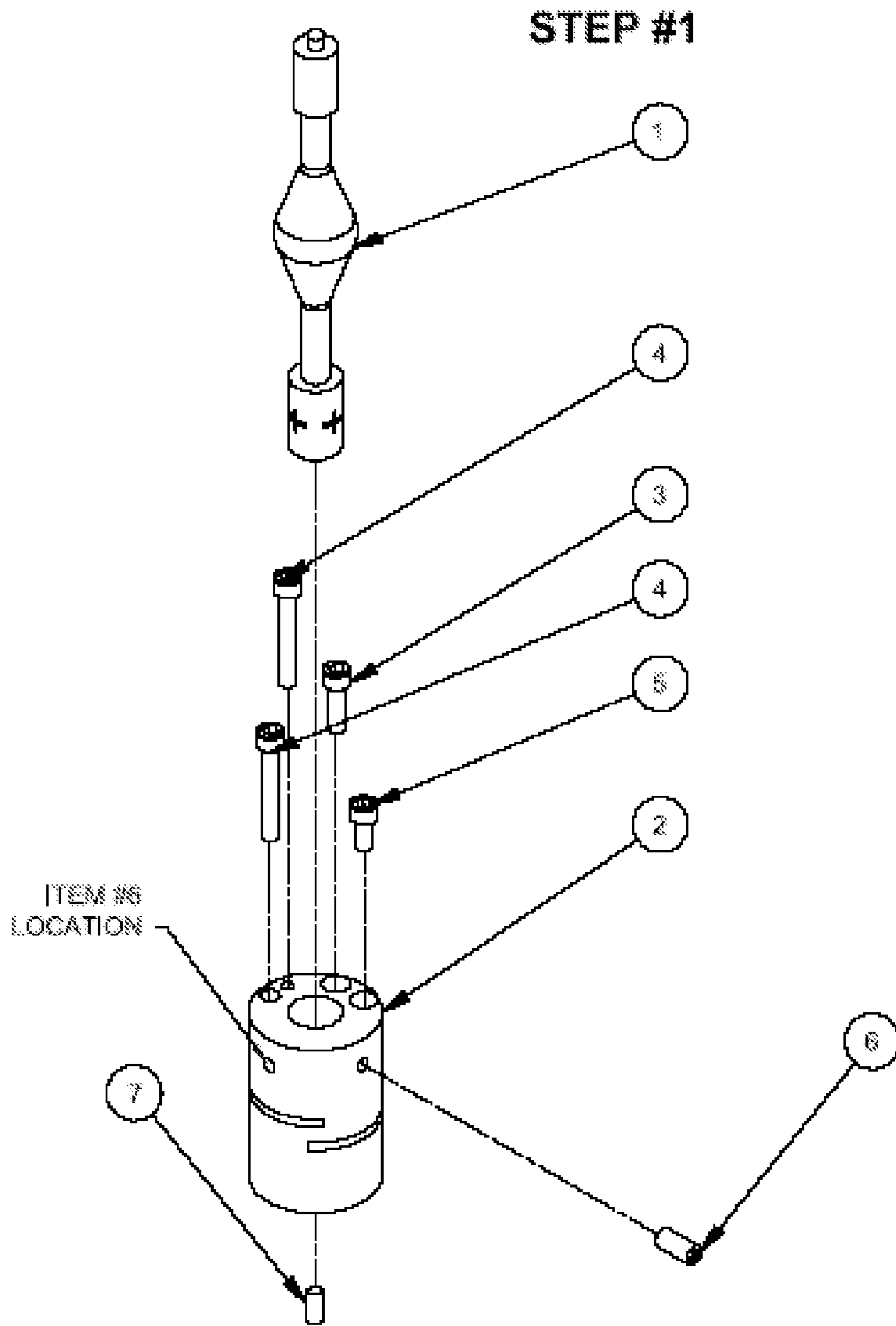


Figure 8

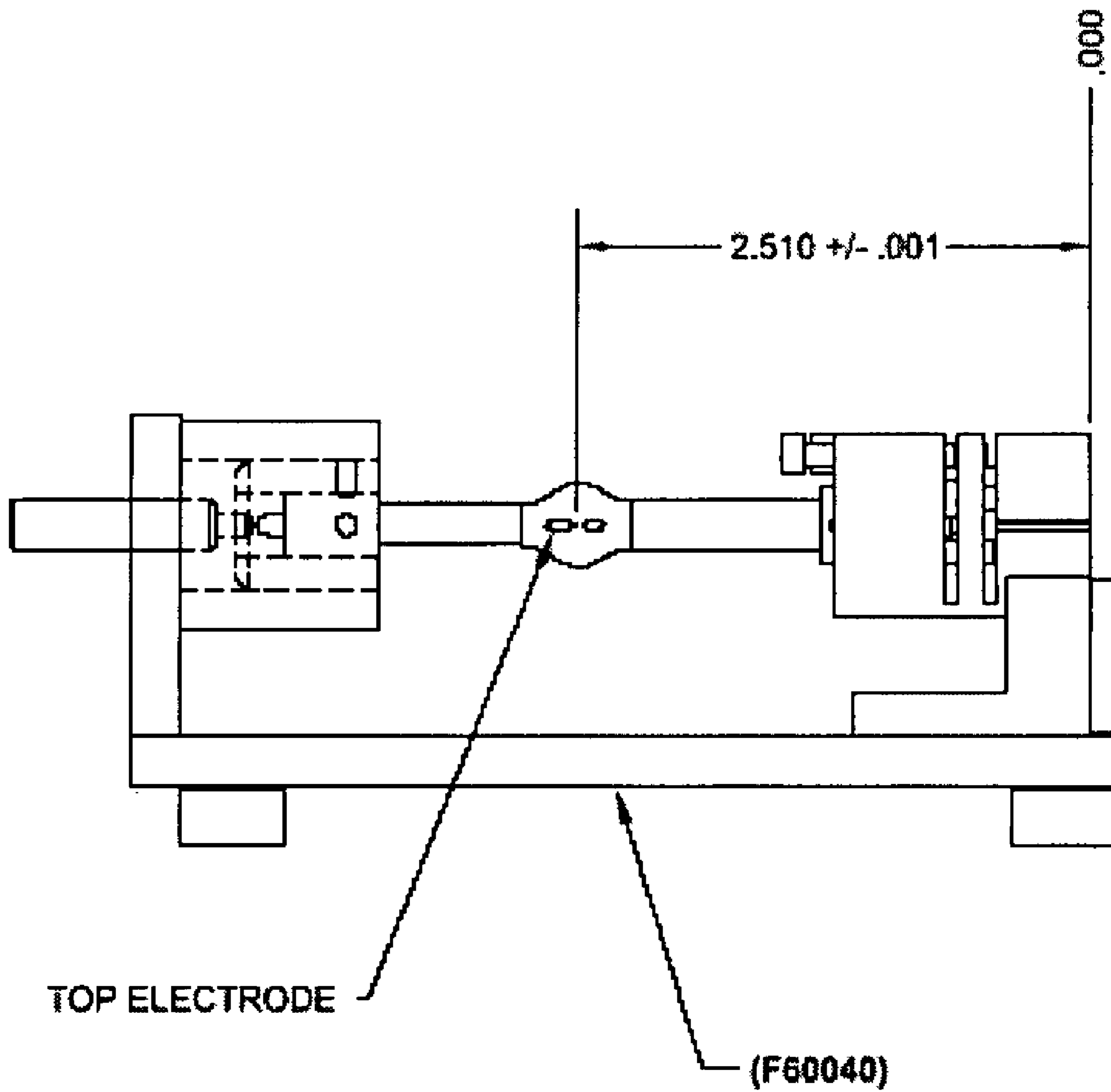
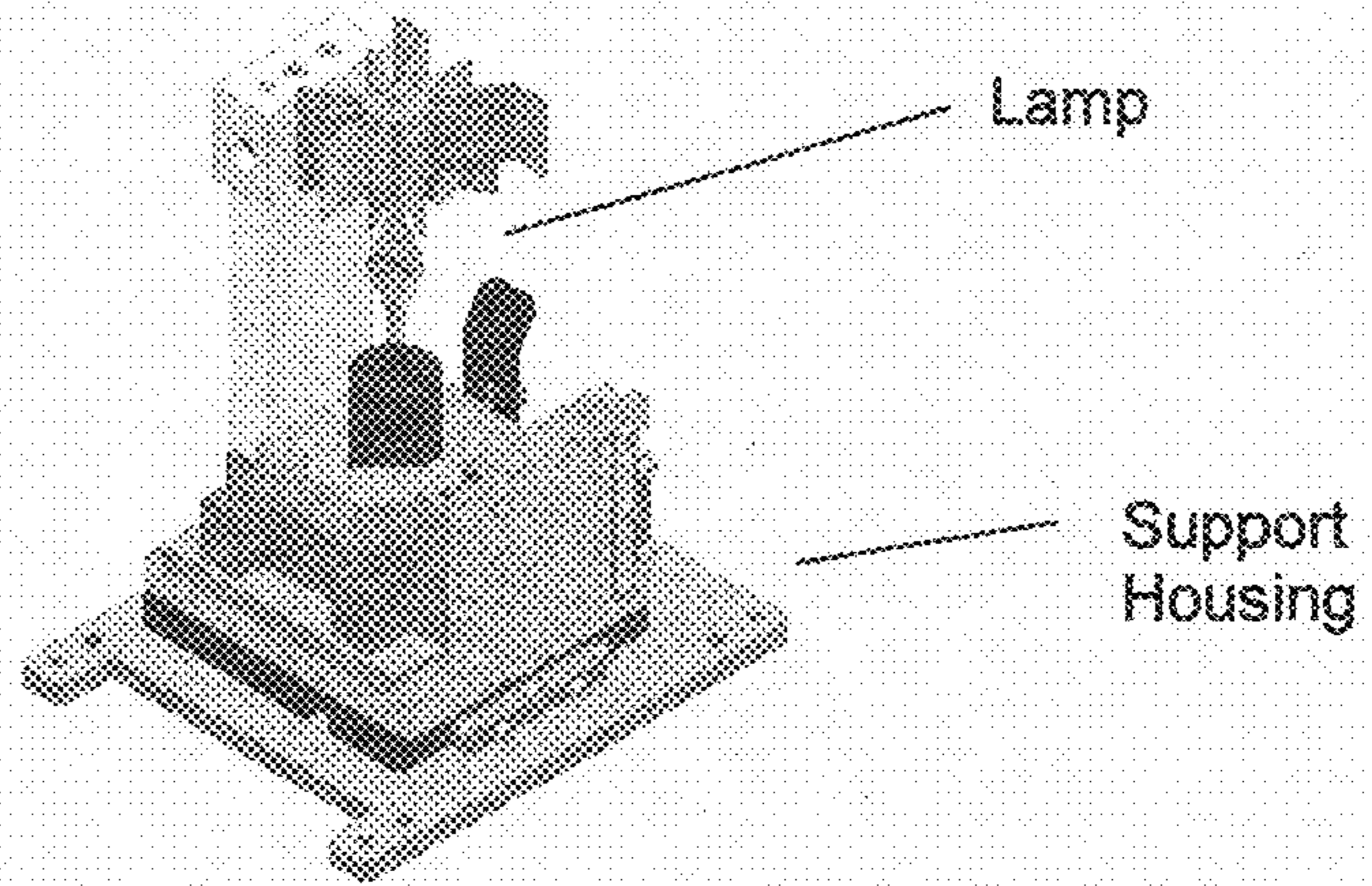
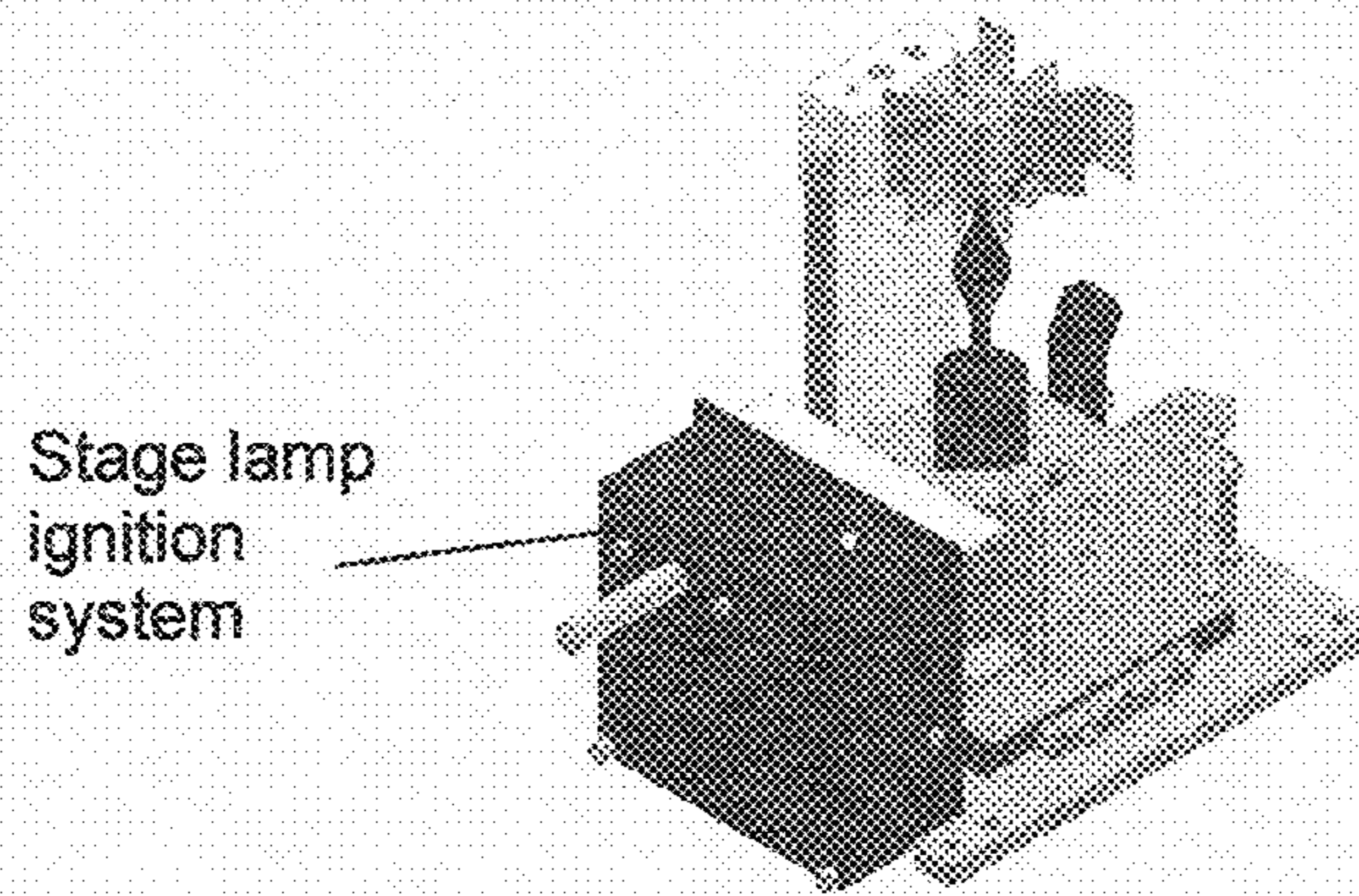


Figure 9

Panel A:



Panel B:



Panel C:

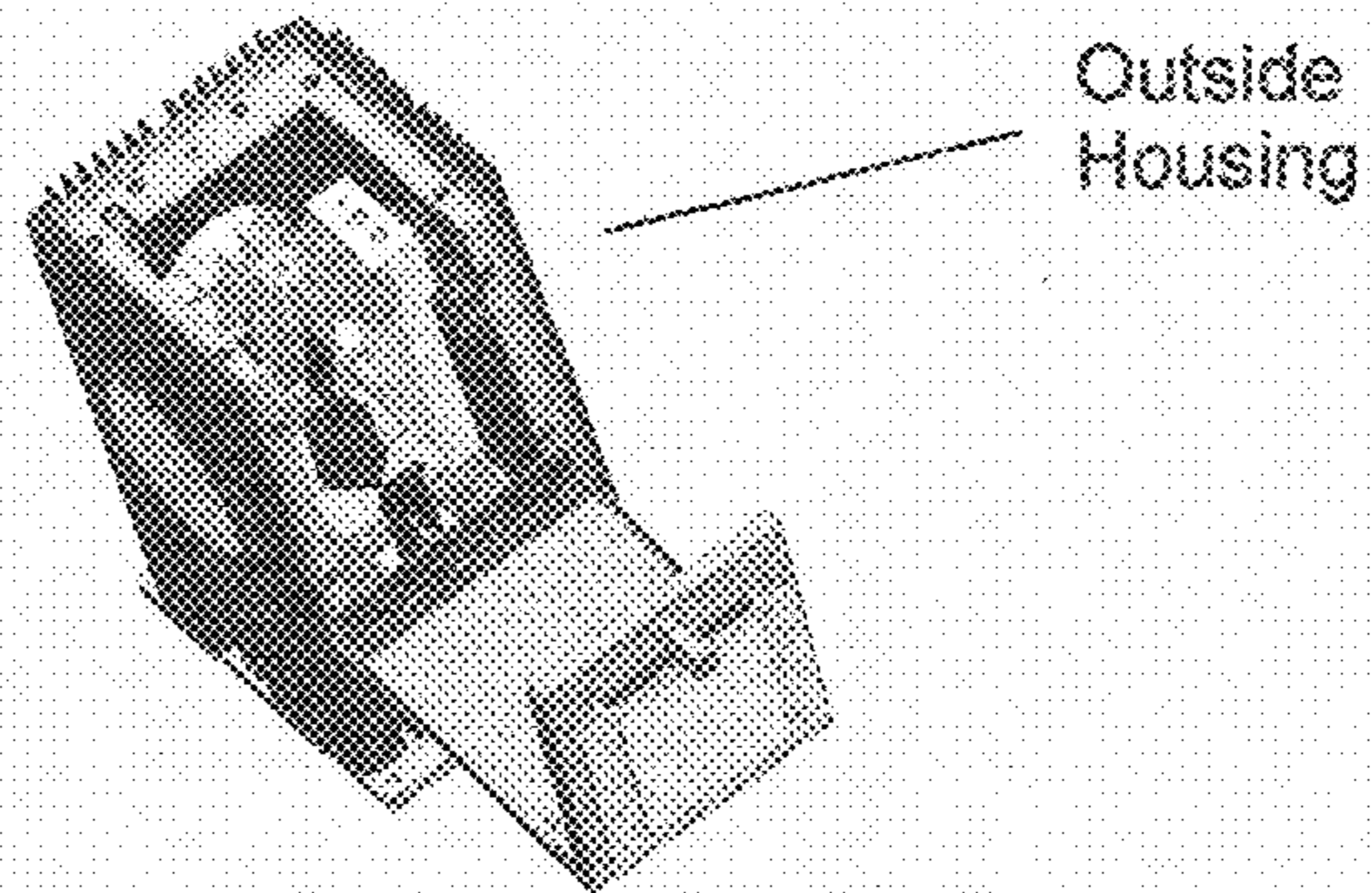


Figure 10

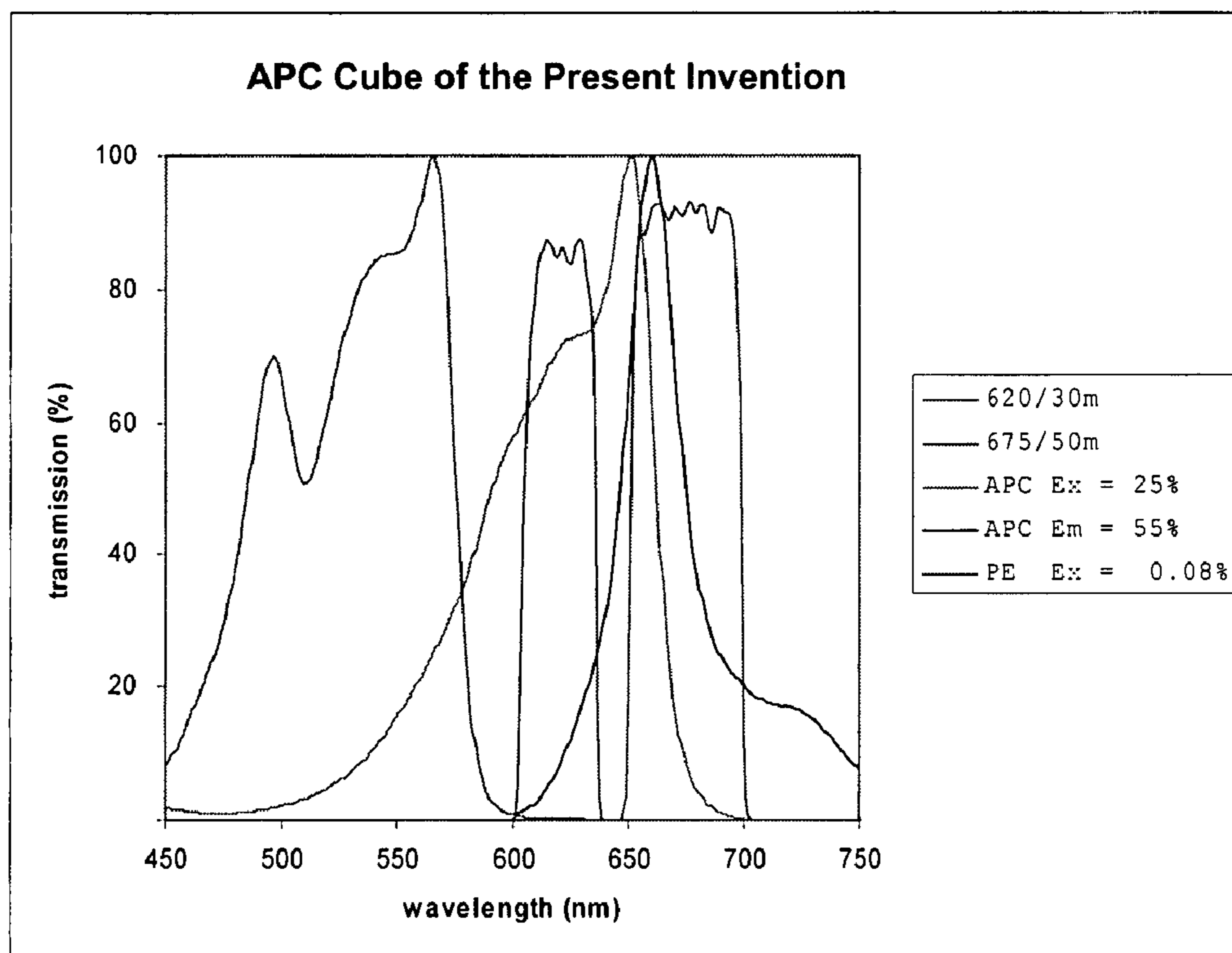
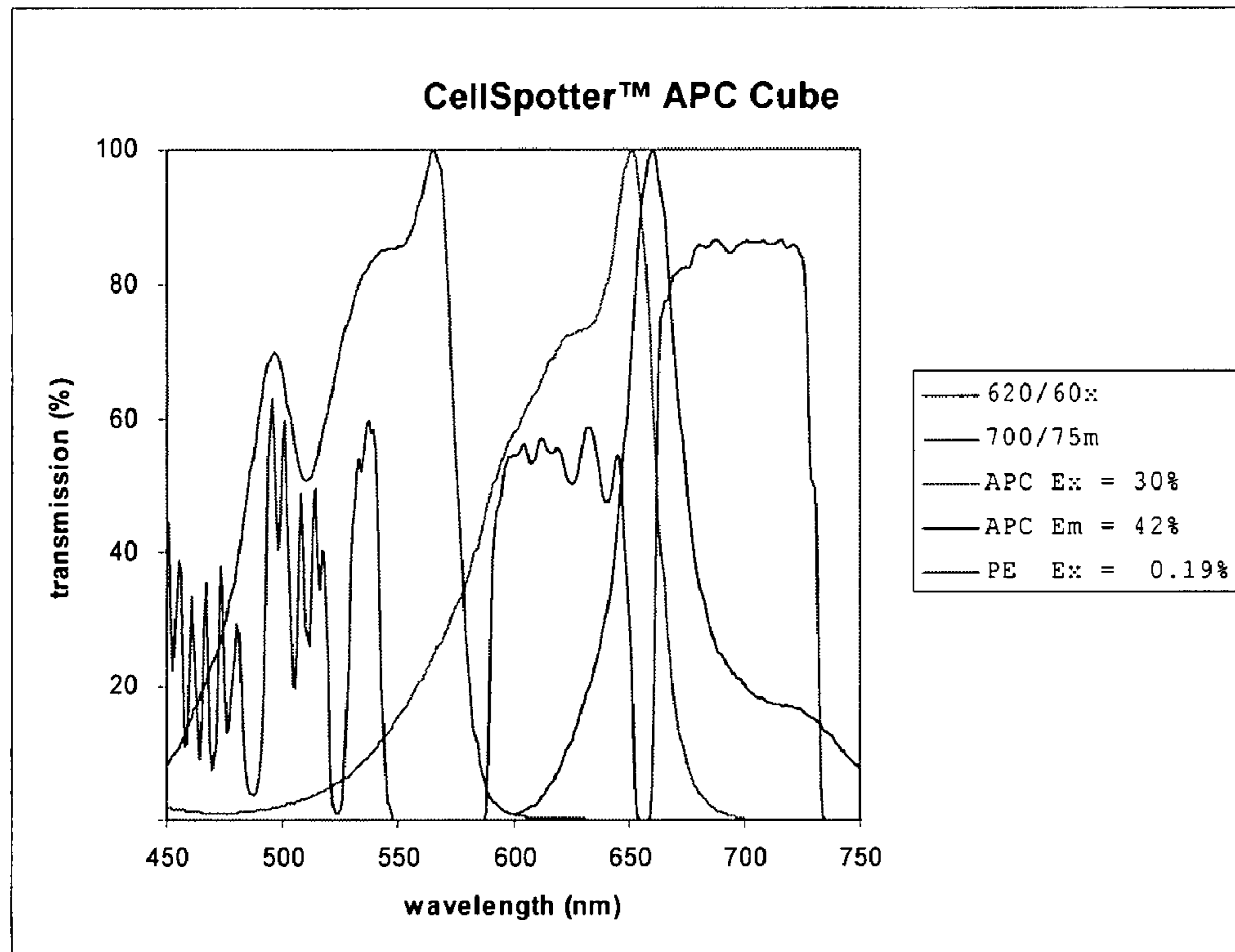
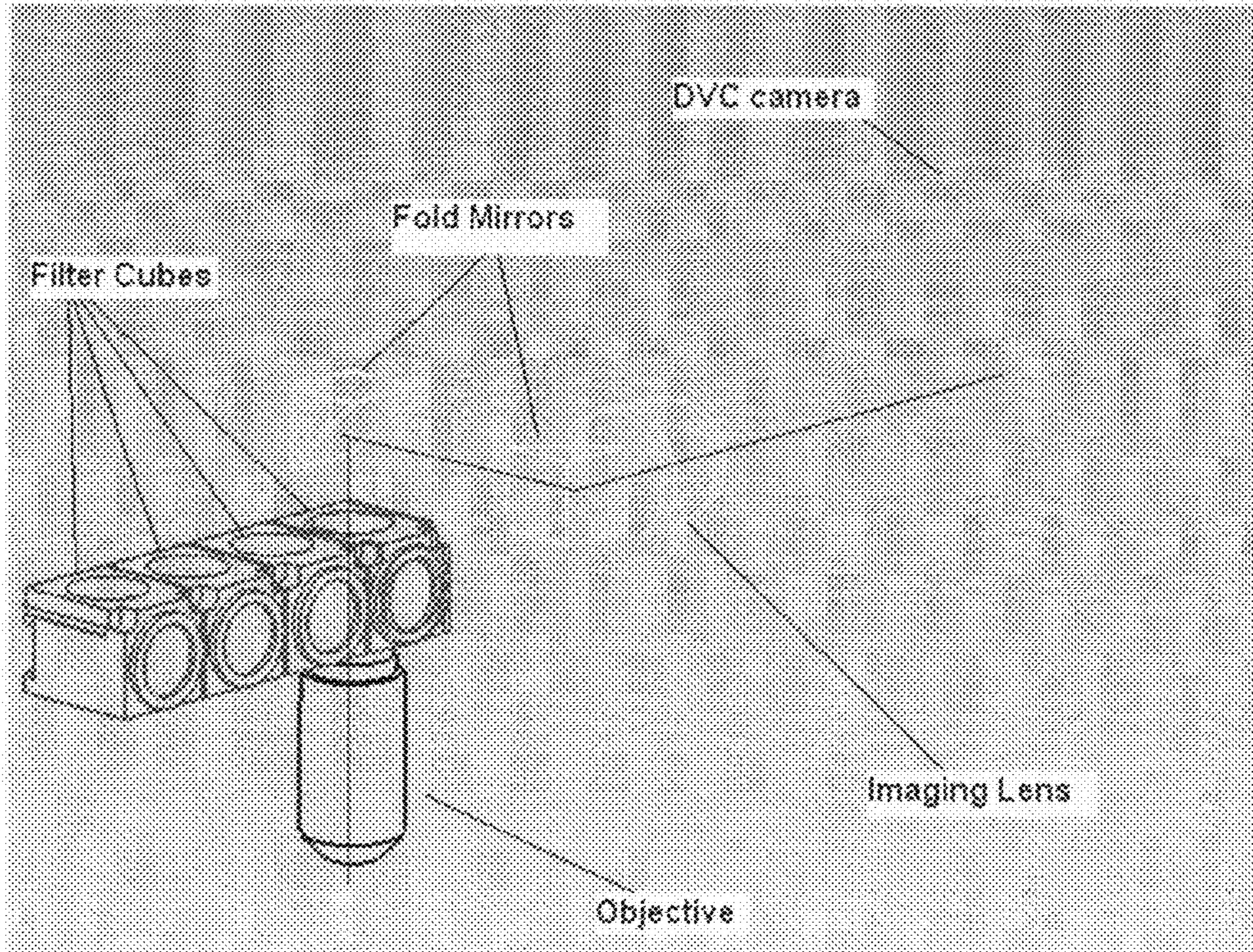
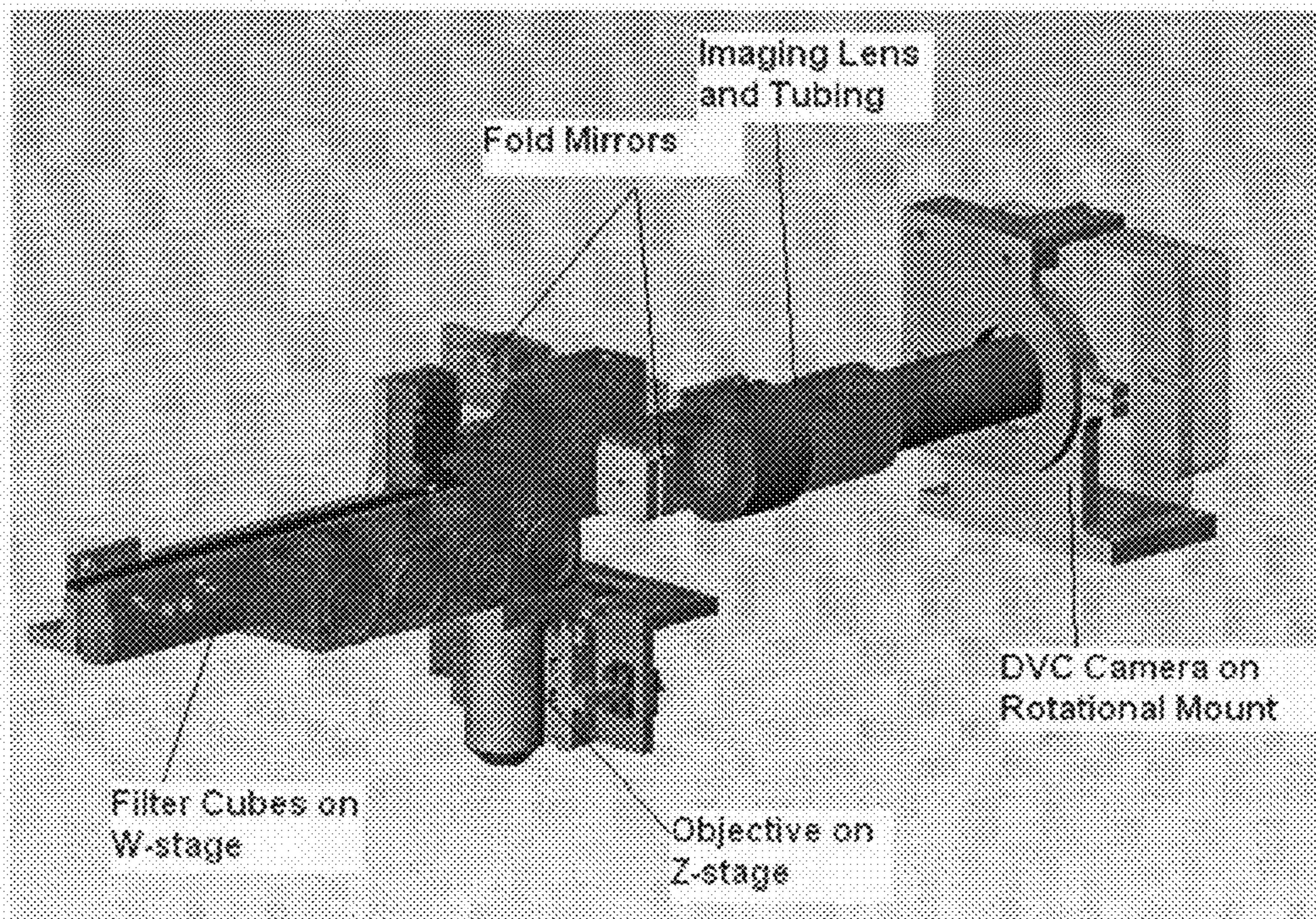


Figure 11

Panel A



Panel B



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**DIAGNOSTIC IMAGING DEVICE FOR THE
ANALYSIS OF CIRCULATING RARE CELLS**

PRIORITY INFORMATION

This application claims priority under 35 USC 119(e) to U.S. Provisional Application Nos. 60/602,504, filed 17 Aug. 2004; 60/645,683, filed 21 Jan. 2005; and 60/648,477, filed 31 Jan. 2005.

FIELD OF THE INVENTION

This invention relates generally to the field of automated sample imaging, and more particularly to image cytometry of isolated rare cells from body fluids by an automated device for analysis relating to the diagnosing, monitoring and managing of specific diseases, particularly cancer.

BACKGROUND ART

Tumor cells are often present in blood of carcinoma patients as rare cells at very low frequencies (<10 cells per ml). These levels may provide clinically useful information. However, the laborious procedures required to detect and quantify the presence of circulating tumor cells and the statistics of low numbers introduce a high level of variability in the results. The system of this invention improves upon imaging devices used in a clinical laboratories for rare cell detection in fluid samples such as blood samples (i.e. circulating rare tumor cells of epithelial origin).

Generally, these rare cells are targeted by labeling and separating from blood by magnetic means. The captured cells are fluorescently labeled to permit detection and differentiation from non-target cells and control cells. CellSpotter® is a semi-automated fluorescence microscope that enumerates and differentiates between immuno-magnetically selected cells based upon fluorescence signals from cells in the blood sample.

A blood sample, suspected of containing the targeted rare cells, is preprocessed by enriching the desired rare cells. Enrichment is obtained by linking antibodies, specific for the target cell, to magnetic particles. This immunomagnetic complex is combined with the blood sample in the presence of a magnetic field, providing for the immunomagnetic capture of the target cells. After obtaining an enriched blood fraction containing the target cell, fluorescent reagents are added for subsequent imaging. Enriched cells are placed into a viewing cartridge which then is placed into a magnetic device that directs magnetically labeled cells in the sample to an optically clear planar surface of the chamber for immuno-magnetical alignment and image analysis. The novel sample chamber (U.S. application Ser. Nos. 10/074,900 and 10,303,309) used in the present invention and the Cell Spotter® system are incorporated in the following patents and co-pending applications; U.S. Pat. No. 5,186,827, 5,698,271, 6,120,856, 6,551,843, U.S. application Ser. Nos. 09/702,188, 10/449,355. The magnetic device and chamber are then placed on a fluorescence microscope equipped with a computer controlled filter selector and digitally controlled X-Y-Z stage. Images are acquired by scanning the sample cartridge with 4 different fluorescent filter sets. The acquired images are processed by automated software analysis to compile data containing images of the target cells. Individual frames are viewed and manually selected for target cells. The analysis is complete with a presentation of a gallery of images containing all selected target cells.

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When incorporated into a clinical laboratory, the system can be used to examine circulating rare cells associated with clinical disease. For example, multi-center prospective, longitudinal clinical trials can be readily established whereby the number of circulating tumor cells (CTC) are correlated with disease progression in patients having metastatic breast cancer (U.S. Pat. Nos. 6,365,362; 6,645,731; U.S. application Ser. No.10/079,939).

Thus, the principal concepts of the present invention stem from laboratory diagnostic equipment systems assessing the detection and enumeration of cells in biological specimens, together with microscope systems for observing the microstructure of a cell under a desired magnification.

Microscopy technology employing stepper motor driven stages for imaging areas larger than the microscope field of view has limited clinical utility. For example, the achievable speed of image acquisition in image cytometry analysis and the overall lab bench space required for the CellSpotter® System limits its practical clinical use. Currently, image acquisition is accomplished by moving a slide or sample cartridge through the field of view in a boustrophedonic step-by-step motion. A boustrophedonic motion is a motion whereby two consecutive lines along the fastest axis are scanned in opposite directions. One image is taken for each step. When multiple fluorescent images of the same sample are obtained, the filter cube is changed and the boustrophedonic motion is repeated after completion of each pass. This process is repeated until an image is acquired for all positions and all colors, completing a color-by-color scan. Both the movement of the filter cube and the sample is accomplished by stepper motor stages with servo feedback, severely limiting the acquisition speed. Moving a filter cube to the next takes approximately 2 seconds with an additional 0.5 seconds to step between two consecutive positions. Thus, the total acquisition time consists of the total motion time plus the total image acquisition time, making acquisition the predominant time spent on motion with imaging systems like CellSpotter®.

Because the prior art employs stepper motor technology for driving microscope stages which is limited to a maximum driving frequency on the stepper motors, there is a need to develop new devices to improve upon large area image acquisition time. Furthermore, moving the sample (or cartridge) can cause the cells to move with the inertia of the fluid. Any motion of a cell could cause the acquisition software to misinterpret the images and count one cell as two with unpredictable identification.

Another issue with the prior art is that during stage motion the sample is continually being illuminated. Consequently, fluorescent dyes used in cell labeling will undergo bleaching. Phycoerythrin (PE) is a dye commonly used in cell labeling, but very sensitive to bleaching. Reducing the time spent on motion will reduce the extent of sample bleaching.

The equipment encompassed in CellSpotter® technology includes a sample chamber (U.S. application Ser. Nos. 10/074,900, 10/303,309, and U.S. Pat. No. 5,993,665) is placed into a magnetic device (U.S. Pat. No. 5,985,153) that directs magnetically labeled cells in the sample to an optically clear planar surface of the chamber. The magnetic device and chamber are then placed on a fluorescence microscope equipped with a computer controlled filter selector and digitally controlled X-Y-Z stage.

The present invention is an improvement to the CellSpotter® automated diagnostic system to provide rapid sample analysis using multiple fluorescent indicators. The imaging device is further improved by condensing the components into a simple box shape for convenient placement on most

clinical laboratory bench tops. This providing a practical configuration in automated laboratories that lack substantial amount of space.

SUMMARY OF THE INVENTION

The present invention provides apparatus and methods to an imaging system for automated operation in a clinical laboratory, improving upon diagnostic imaging devices for rare cell detection used in clinical laboratories. The device of the present invention is a compact and self-contained unit, designed to fit on a laboratory bench top with minimum space in a simple, exterior box-like design. The illumination and collection optics are structured to provide a clinical laboratory technician easy access and use in the analysis of large numbers of patient samples.

Further, stepper motor technology, used in changing the filter cubes and stage movement, has been replaced with nanomotion motors in a closed feedback loop. These movements are critical if an image is to be developed from multiple fluorescent detectors or dyes whereby more than one filter cube is required. More specifically the motion time for each cube is reduced from 2 to 0.2 seconds for two consecutive filter cubes. The time spent moving between two consecutive positions is reduced from 0.5 to 0.1 seconds. Because the area needed for image acquisition is larger than the system field of view, the acquisition of an entire image requires acquisition of images at several different positions. Nanomotion motors reduce the time period required for these functions and limits the fraction of the total acquisition that is time spent on motion. The majority of time will then be spent on acquisition of the image itself, instead of time spent moving the sample and/or cubes. Further with the total acquisition time drastically reduced, the bleaching of the sample, especially with PE, is reduced. The substantial reduction in acquisition time allows for a position-by-position scan of the entire image. With a position-by-position scan, images are acquired for all filter cubes before moving on to the next position in a boustrophedonic motion. Any possible movement of the image during a color-by-color scan is eliminated.

The automated fluorescent optical imaging device of the present invention further provides a prealigned bulb and method for installation/use in the enumeration of fluorescently labeled circulating tumor cells (CTC) that are immuno-magnetically selected and aligned. This invention provides for the standardization of the lamp housing stage through the establishment of a master lamp/stage unit. This master lamp is seated within an adjustment collar as shown in the drawings. The collar has machined gaps which are used to microalign the bulb filament. Thus the collar is used to ensure exact alignment (for X-Y-Z orientation) of the electrodes with respect to any instrument unit. Once aligned, the collar-bulb unit is inserted into a V-block structure for final support. A further improvement to any subsequent shifting in the alignment is the use of a braided wire as a flexible connecting lead. These combined improvements allow for a prealigned bulb to be used in any corresponding imaging unit. This reduces the costs of aligning individual units at their respective locations and provides for efficient installation.

Thus, this automated rare cell imaging device improves upon the speed of sample analysis. The system's compact design in a clinical diagnostic laboratory enables enumeration and identification of target cells for targeting the clinical status of diseases such as metastatic cancer. The pre-aligned bulb structure provides rapid, convenient maintenance. Together, these concepts aid in the detection and quantification of target cells in a sample specimen, such as but not

limited to, screening and detection in early stage cancer, monitoring for disease remission in response to therapy and selection of more effective dose regimens or alternative therapies in case of relapse.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1: The overall design of the benchtop profile. (A) Exterior view of box-like unit. (B) Internal view of system components.

FIG. 2: Modified stage for communication between MagNeSt™ and the Imaging System.

FIG. 3: Diagram showing linear motor and linear encoder relationship to provide positional accuracy.

FIG. 4: Diagrammatic representation of the axis direction of movement for the filter cube (W), objective (Z), and cartridge (X and Y).

FIG. 5: Diagram showing illumination path in relation to instrument components.

FIG. 6: Diagram of illumination path showing light path.

FIG. 7: Diagram of the pre-aligned structure showing the lamp (1) with support collar (2). Adjustment screws (3), (4), and (5) are on the top portion of the collar while adjustment screw (6) is located on the lateral surface and adjustment screw (7) is on the bottom.

FIG. 8: Schematic representation of the alignment fixture-F60040 with lamp with electrode in position.

FIG. 9: Diagram of the complete support apparatus for the lamp. Panel A shows the support apparatus with the lamp. Panel B shows the apparatus with the stage lamp ignition system in place. Panel C shows the apparatus with the outside housing.

FIG. 10: Comparison of CellSpotter APC Cube with the APC Cube of the Present Invention.

FIG. 11: Diagram of imaging path with direction of light in relation to camera, lens, mirrors, cubes and objective (Panel A). Imaging path shown with apparatus in position (Panel B).

DETAILED DESCRIPTION OF THE INVENTION

The apparatus of this invention improves upon current CellSpotter® technology to provide a more robust application with less user intervention in a device that is more conducive to a clinical laboratory environment.

First, the overall layout is a non-microscope type design to provide a compact benchtop profile for efficient space management in the laboratory (see FIG. 1). The system is encased in a simple, streamline boxed unit to avoid connecting-cable clutter and to discourage access by laboratory personnel (FIG. 1A). The internal components are designed to provide efficient illumination and collection optics in a confined space (FIG. 1B)

Second, improvements to individual components provide more versatility. The unit can scan an individual sample for multiple analyses. Further, the unit is expandable to include multiple analyte specific reagents (ASR) and the associated software algorithms. MagNest™, the magnetic device, communicates to the stage through a data button interface (see FIG. 2). The stage is improved in both accuracy (see FIG. 3) and precision. The platform motion functions through a motion controller (Galil), piezoelectric motor (Nanomotion), and position encoders (Renishaw). This provides 0.2 micron resolution on the X, Y, Z plane and with the filters (W), as shown in FIG. 4. The optical components include an Oriel Lamp Assembly with an integrated Nikon lens which is operator adjustable. The lens system has very few components to reduce the amount of light lost in surface reflections

TABLE I-continued

All colors one position before moving to the next position

	Start	Position 1				Position 140				Return
		DAPI	PE	FITC	APC	DAPI	PE	FITC	APC	
Initial/ final move	▲					▲				▲
Move xy	▲				■	▲			■	▲
Move Filter	▲	■	■	■	■	▲	■	■	■	▲
Acquire Image	▲	■	■	■	■	▲	■	■	■	▲
Transfer Image	▲	■	■	■	■	▲	■	■	■	▲

The time spent acquiring individual images is the same in both methods, but different for different dyes, 0.11 sec for DAPI, 0.22 sec for PE, 0.11 sec for FITC, and 0.66 sec for APC. Comparison of the color-by-color method with the position-by-position method shows that the main difference is that the total time spent is affected by the time to move the sample in the x-y direction.

The total color-by-color time is modeled by the following:

$$T_{color-by-color} = T_{init} + 140 \times T_{acquisition} + 560 \times (T_{move\ xy} // T_{transfer}) + 4 \times T_{move\ filter} + T_{final} \quad (1)$$

For the position-by-position the model is given by the following:

$$T_{position-by-position} = T_{init} + 140 \times T_{acquisition} + 140 \times (T_{move\ xy} // T_{transfer} // T_{move\ filter\ 4-1}) + 420 \times (T_{move\ filter\ 1-2/2-3/3-3/3-A} // T_{transfer}) + T_{final} \quad (2)$$

where (A//B) means A if (A>B) and B if (A≤B).

Assuming the following values for CellSpotter® system, $T_{init} + T_{final} = 60$ seconds, $T_{acquisition} = 1.1$ seconds, $T_{transfer} = 0.11$ seconds, $T_{move\ xy} = 0.5$ seconds, $T_{move\ filter} = 2$ seconds, $T_{move\ filter\ 4-1} = 6$ seconds, the resultant total acquisition time is 500 seconds (8 minutes 20 sec) for the color-by-color approach and 1900 seconds (31 minutes 40 sec) for the position-by-position approach.

In the present invention, these times are the following; $T_{acquisition} = 0.6$ seconds, $T_{transfer} = 0.11$ seconds, $T_{move\ xy} = 0.1$ seconds, $T_{move\ filter} = 0.2$ seconds, $T_{move\ filter\ 4-1} = 320.5$ seconds.

The higher QE of the ICX285 chip reduces $T_{acquisition}$ time while the reduction in time for $T_{move\ xy}$ and $T_{move\ filter}$ are due to the nanomotion stages and filter changer, respectively. The reduced times result in total times of 200 seconds (3 minutes 20 seconds) and 300 seconds (5 minutes) for color-by-color and position-by-position approach, respectively. Total acquisition times for both approaches are substantially reduced. So while the color-by-color is still faster, the difference is small enough to make the position-by-position more practical. In fact, the reduced acquisition time allows imaging with PE without significant interference from bleaching.

A further advantage of the position-by-position approach is that the cells stay aligned.

Finally, the present device is designed to provide easily replaceable units in the field to allow rapid, low cost service calls. These units include power supplies, X-Y stage assembly, Z-stage assembly, filter (W) stage assembly, servo amplifiers (4), CCD camera, imaging and illumination optics assembly, objective, filter cubes, lamp and housing, computer and peripherals, shutter/ND filter assembly, air filters, main circuit board and cables.

Safety provisions include an enclosure interlock closing system shutter. The lamp housing interlock disables the lamp supply. UV filter safety glasses are supplied with the instrument. The instrument can operate in the US, Europe and Japan without switching any components.

It is to be understood and appreciated that these improvements are only illustrative of the many additional potential applications of the apparatus and method that may be envisioned by one of ordinary skill in the art, and thus are not in any way intended to be limiting of the scope of the invention.

What is claimed is:

1. A clinical laboratory automated imaging system for detecting rare cells from a biological sample comprising:
 - a. an illumination system having an optimum illumination path whereby said path allows for compact design;
 - b. an X-Y-Z stage assembly motioned through piezoelectric motors and having a platform to position a magnetic device and sample cartridge for imaging;
 - c. a fluorescent detection system having at least one APC filter cube motioned through piezoelectric motors wherein said APC cube reduces PE bleed-over by approximately 50% while maintaining APC sensitivity; and
 - d. a computer with related peripherals wherein elements a, b, c and d of the system form a compact and self-contained structure in an optimized size for use on a laboratory bench top.
2. The illumination system of claim 1 whereby said illumination system consists of a CCD camera, illumination optics assembly, objective, filter cubes, lamp with housing, and shutter/ND filter assembly.
3. The illumination system of claim 2 whereby said lamp is pre-aligned.
4. The illumination system of claim 1 whereby said illumination path allows easy access to associated lenses filters and light source.
5. The illumination system of claim 1 with the capability to produce fluorescence excitation wavelengths between 350 to 1000 nanometers.
6. The X-Y-Z stage assembly of claim 1 wherein motion of said stage is through said piezoelectric motor capable of providing 0.2 micron resolution.
7. The detection system of claim 1 wherein said detection system contains a backlight structure for alignment of said sample cartridge.
8. The detection system of claim 1 wherein the chip width is small enough to scan 175 frames per filter.

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- 9.** A method for acquiring multiple images from a sample cartridge by a color-by-color approach comprising:
- a. setting system to an image position;
 - b. moving cartridge sample in the x-y direction corresponding to said position whereby said moving is with a piezo-
electric motor; 5
 - c. positioning a filter cube;
 - d. acquiring said image;
 - e. transferring said image; and
 - f. repeating elements c through e for an x-y position 10
whereby said repeating is for each filter cube wherein at least one filter cube is APC having reduced PE blood-over by approximately 50% while maintaining APC sensitivity.
- 10.** A method for acquiring multiple images from a sample 15
cartridge by a position-by-position approach comprising;

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- a. positioning a first filter cube;
- b. setting system to an image position;
- c. moving cartridge sample in the x-y direction corresponding to said position;
- d. acquiring said image;
- e. transferring said image;
- f. repeating elements b through e for each x-y position; and
- g. positioning another filter cube to complete elements b through f whereby said elements are repeated for each cube and said positioning with a piezoelectric motor, wherein at least one filter cube is APC having reduced PE blood-over by approximately 50% while maintaining APC sensitivity.

* * * * *